PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07K 14/40, C12N 15/31 | A1

(11) International Publication Number: WO 98/34950

(43) International Publication Date: 13 August 1998 (13.08.98)

(21) International Application Number: PCT/US98/02332

(22) International Filing Date: 6 February 1998 (06.02.98)

(30) Priority Data:
60/037,859 7 February 1997 (07.02.97) US

(81) Designated States: CA, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(71) Applicant *(for all designated States except US)*: THE UNIVERSITY OF TENNESSEE RESEARCH CORPORATION [US/US]; 415 Communications Building, Knoxville, TN 37996–0344 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BECKER, Jeffrey, M. [US/US]; 7125 Cresthill Drive, Knoxville, TN 37919 (US). LUBKOWITZ, Mark, A. [US/US]; The University of Tennessee Research Corporation, Microbiology Dept., M409 Walters Building, Knoxville, TN 37996–0334 (US).

(74) Agents: WEISER, Gerard, J. et al.; Weiser & Associates, P.C., Suite 500, 230 South Fifteenth Street, Philadelphia, PA 19102–3838 (US).

(54) Title: ISOLATED CANDIDA ALBICANS OLIGOPEPTIDE TRANSPORTER GENE

(57) Abstract

A *Candida albicans* oligopeptide transport gene, *OPT1*, was cloned from a *C. albicans* genomic library through heterologous expression in the *Saccharomyces cerevisiae* di–/tripeptide transport mutant PB1X–9B. When transformed with a plasmid harboring *OPT1*, *S. cerevisiae* PB1X–9B, which did not express tetra–/pentapeptide transport activity under the conditions used, was conferred with an oligopeptide transport phenotype as indicated by growth on the tetrapeptide Lysyl–Leucyl–Leucyl–Glycine, sensitivity to toxic tetra– and pentapeptides, and an increase in the initial uptake rate of the radiolabeled tetrapeptide Lysyl–Leucyl–Glycyl–[^{3}H]Leucine. The entire 3.8 kb fragment containing the oligopeptide transport activity was sequenced and an open reading frame of 2349 nucleotides containing a 58 nucleotide intron was identified. The deduced protein product of 783 amino acid residues contained twelve hydrophobic regions suggestive of a membrane transport protein. The oligopeptide transporter facilitates targeting of antifungal, especially anticandidal drugs.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

ISOLATED CANDIDA ALBICANS OLIGOPEPTIDE TRANSPORTER GENE

RELATED CASES

This application claims the benefit of provisional patent application Serial No. 60/037,859, filed on February 7, 1997, entitled An Oligopeptide Transport Gene from *Candida albicans*, which is incorporated herein it is entirety by reference.

FIELD OF THE INVENTION

The invention relates to novel nucleic acid sequences encoding peptide transporters, to novel polypeptides and drug delivery systems.

BACKGROUND OF THE INVENTION

Peptide transport, a phenomenon defined as the translocation of peptides across the plasma membrane in an energy-dependent manner, has been well documented in bacteria, plants, fungi, and mammals (for reviews see Becker & Naider, 1995; Payne and Smith, 1994). Upon internalization, peptides are quickly hydrolyzed into their amino acid components to serve as sources of amino acids or nitrogen. In addition to acquiring nutrients from the environment, peptide transport has been shown to play a role in recycling cell wall peptides and in transducing signals for group behaviors such as sporulation and competency in *B. subtilis* and chemotaxis in *E. coli*. Recently it has been proposed that in *Salmonella typhimurium* peptide transporters aid the bacteria in evading the host immune response by transporting membrane disrupting peptides away from the plasma membrane (Parra-Lopez *et al.*, 1993). Similarly, in *Streptococcus pneumoniae* the peptide transporters encoded by *plpA* and the *amiA* loci play a role in virulence by modulating adherence to epithelial and endothelial cells (Cundel *et al.*, 1995).

A family of di-/tripeptide transporters named the PTR (Peptide TRansport) Family has recently been identified. This family is characterized by several conserved motifs, has twelve putative transmembrane domains, and is driven by the proton motive force. Members of the PTR family have been identified in a broad variety of eukaryotes and one prokaryote as well (Steiner *et al*, 1995). Well characterized members of the PTR family are the di- and tripeptide transporters from *S. cerevisiae* (*ScPTR2* , Perry *et al.*, 1994) and from *C. albicans (CaPTR2* , Basrai *et al.,* 1995). Both *CaPTR2* and *ScPTR2* have been shown to be regulated by nitrogen source and inducible by micromolar amounts of amino acids; their encoded proteins have broad substrate specificities with a preference for peptides containing hydrophobic residues (Basrai *et al.*, 1992; Island *et al.*, 1987). Prior to the establishment of the PTR family, all peptide transporters cloned were from prokaryotes and were members of the ATP Binding Cassette (ABC) Superfamily (Higgins, 1992). Recently, transporters from the PTR family have been identified in the prokaryote *Lactococcus lactis* (Hagting *et al.*, 1995). However, in eukaryotes all peptide transporters thus far identified are members of the PTR family.

In addition to the di-/tripeptide transporter *(CaPTR2)* in *C. albicans,* three observations indicated the existence of another distinct peptide transport system. The first observation was that mutants resistant to the toxic peptide analogs bacilysin, polyoxin, and nikkomycin Z (all demonstrated substrates of the di-/tripeptide transport system) were able to transport tetra- and pentapeptides at wild type levels, and, conversely, mutants resistant to various toxic tetrapeptides were able to transport dipeptides at wildtype levels (Payne and Shallow, 1985; Milewski *et al.*,. 1988; McCarthy *et al.*, 1985). Secondly, peptide uptake experiments with radiolabeled compounds and chromophoric substrates demonstrated that dipeptides did not compete with tetra- and pentapeptides for entry into the cell, and *vice versa* tetra- and pentapeptides did not compete with labeled dipeptides (Milewski *et al.*, 1988; McCarthy *et al.*, 1985; Yadan *et al.*, 1984).. Thirdly, sensitivity to toxic di- and tripeptides was influenced by nitrogen source and micromolar amounts of amino acids while sensitivity to toxic tetra- and pentapeptides was not regulated by similar

means (Basrai *et al.*, 1992). The invention described herein relates to the cloning of a novel oligopeptide transporter from *C. albicans* that does not code for an ABC- or PTR-type transporter.

SUMMARY OF THE INVENTION

The invention relates to a new transporter system: a system to transport oligopeptides as opposed to lower peptides. The invention relates to an oligopeptide transporter competent to transport higher oligopeptides, especially tetra- and pentapeptides. The expression of transport activity is evidenced in a heterologous host suggesting that the transporter is an integral membrane transporter. The ability to transport peptides of a size larger than di/tripeptides is highly significant in that it will permit the delivery of greater variety of biological molecules in molecular structure and size into the selected target.

Furthermore, there is provided a novel peptide transport gene from *Candida albicans* through heterologous expression in *Saccharomyces cerevisiae*, which encodes an oligopeptide transporter *OPT*, which is different from the previously identified family of di-/tripeptide transporters named the PTR (Peptide Transport) family. The gene encoding *OPT*1 appears to constitute the first identified member of a new family of oligopeptide transporters.

The gene sequence revealed the presence of two ORFs separated by a type II intron, and encoding a hydropholic protein of 783 amino acids with an apparent molecular mass of 88kDa and a pI of 7.1. The size and hydrophobic nature of the predicted protein of *OPT*1 suggest a membrane/bound protein with at least 12 putative transmembrane domains of 20-24 amino and residues. Findings made in connection with invention indicate that *OPT*1 is not a member of the PTR or ABC families of membrane transporters.

Peptide utilization mediated by *OPT*1 showed its ability to mediate the uptake of Lys-Leu-Gly (KLG), Lys-Leu-Leu-Gly (KLLG), Lys-Leu-Gly-Leu (KLGL) and Lys-Leu-Leu-Leu-Gly (KLLLG).

Various eukaryotic transformants of the yeasts are made available by the invention.

The invention provides peptide transporters as a means to facilitate the uptake of otherwise nonpermeating biologically active molecules of medical significance, such as antifungal compounds.

Hetofore it was known that *C. albicans* can transport and utilize small peptides. The invention allows using *C. albicans* with the novel oligopeptide transporter for the uptake of peptide-drug adducts. In the search for effective antimicrobial drugs, substances are often found that display toxicity towards intracellular targets when tested in cell-free systems, but are inactive with intact organisms. Frequently this occurs because the potentially toxic agent is impermeable. The invention provides a drug delivery system whereby a toxic moiety is linked or otherwise carried by a molecule which will be taken up and actively transported through a specific permease for delivery to the target. But for the membrane transporter system described herein, such drug delivery system are known. For instance, the uptake by dipeptides containing N^3-(4-methoxyfamaroyl)-L-2,3-diamino-propanoic acid (FMDP) has been extensively studied. Literature references dealing with such drug delivery designs are incorporated herein by reference. For instance, it is known that N-acylation can stabilize the carrier toxic agent conjugate to amino peptidase activity. See Peptide Base Drug Design, Becker and Naider cited herein.

Infections attributable to *C. albicans* are wide spread. The oligopeptide transport system of the invention is useful to deliver anticandidal drugs carried (conjugated or linked or associated) and taken up by the peptide, delivered to the transporter which will deliver it through the membrane to the target.

The oligopeptide transporter of the invention will promote the more effective delivery of anti candidal drugs into organisms infected by *C. albicans*. Such drugs can be molecules like toxic peptides carried, if necessary by a carrier, or molecules that mimic or are similar in character to the peptides, like peptido-mimetics. The invention also provides for the delivery of the *OPT*1 gene into a mammalian target call where it will express the oligopeptide transporter, thereby facilitates the targeting of the desired drug.

The ability to use the transport system encoded by the OPT gene will allow delivery of toxic agents specifically into cells or organisms expressing this gene. Thus, if pathogenic fungi express such a gene in an infected human host that is not capable of expressing this gene, then antifungal agents can be designed to kill the invading pathogen without having any adverse effects on the human host. Such non-toxic antifungal agents are the major goal of all pharmaceutical companies with antifungal drug programs. Currently, extensive research is carried out throughout the world in the search for antifungal drugs.

The *OPT* gene of C. albicans opens the way for gene discovery of a family of plant oligopeptide transport genes. Genes in the family represented by the *OPT* gene of C. albicans have been found to date only in other fungi, and some potential homologous genes have been noted in the plant EST database. Using fungi as the heterologous hosts for testing oligopeptide transport ability, full-length plant genes should be uncovered and characterized by techniques used in this invention to clone di-/tripeptide plant transporters. Thus, oligopeptides might be useful agents as herbicides or growth stimulators depending on the chemical constituents of a modified oligopeptide. Delivery of such oligopeptide-based analogs to plant cells via the oligopeptide transport system would allow specificity in targeting. Also, uptake into the plant cell would occur in large quantities due to the ability to transport systems to concentrate substrates intracellularly to high levels.

Other embodiments will become apparent from the description that follows.

DESCRIPTION OF THE DRAWINGS

Figure 1 is a partial restriction map of the 3·8 kb fragment from p*OPT*1. The location and orientation of the ORF are indicated as well as the location of the probe used in Southern blots. Restriction sites are as follows: B, *Bst*XI; H, *Hin*cII; P, *Pvu*II; Ba, *Bam*HI; K, *Kpn*I.

Figure 2 is a southern blot. Analysis of hybridization of a probe of *OPT*1 to genomic DNA isolated from *C. albicans* SC5314 was performed as described in Methods. Lanes: 1, *Hin*c11 digest; 2, *Pvu*ll-*Bam*Hl digest; 3, *Pvu*ll-*Kpn*l digest. Size markers are in bp.

Figure 3 is a nucleotide and predicted amino acid sequences of *OPT*1. The predicted amino acids are italicized and numbered to the left of the figure while nucleotides are numbered to the right. The 5' and 3' splice sites as well as the conserved branch point of the intron are boxed. The codon CUG (CTG in the DNA) encodes serine not leucine in *C. albicans* (Omaha *et al.*, 1993).

Figure 4 is a comparison of oligopeptide transporters. The proteins Optl, lsp4, SCYJL212C and YSCP9677 were aligned using the program PileUp. Conserved residues are in upper case and denoted as the consensus, while nonconserved residues are in lower case. The amino acids in each respective protein are numbered to the right.

Figure 5 is a toxic peptide inhibition assay. Sensitivity to the ethionine-containing peptides KLLAEth (1) and KLLEth (3) on a 0·1% proline medium was determined as described in Methods. (A) PB1X-9B(pRS202); (b) PB1X-9B(pOPT1).

Figure 6 is a peptide transport assay. Accumulation of KLG-[^{3}H]L was measured over a 12 min time course as described in Methods. *S. cerevisiae* PB1X-9B harbouring pRS202 (●) or p*OPT*1 (▽) and *C. albicans* SC5314 (○) were grown in SC-Ura medium with either ammonium sulfate (a) or 0·1% proline (b) as a nitrogen source.

Figure 7 is a peptide transport competition experiment. Accumulation of KLG-[^{3}H]L (○) was measured in the presence of a 10-fold molar excess of the competitors L (●), KL (▽), KLG (▼), KLLG (■) over a 12 min time course.

The following experimental results and examples are not intended to be limiting but rather illustrative of the invention.

DESCRIPTION OF RESULTS OF THE PREFERRED EMBODIMENT

Cloning of an Oligopeptide Transporter

Recently, the cloning of di- and tripeptide transporters of *Candida albicans (CaPTR2)* (BasraiÊ*et al.*, 1995) and *Arabidopsis thaliana (AtPTR2-A* and *AtPTR2-B)* (Steiner *et al.,* 1994; Song, *et al.,* 1996) (U.S. Patent No. 5,689,039) through heterologous expression in *Saccharomyces cerevisiae* has been reported. Unlike *C. albicans*, *S. cerevisiae* has been found to transport only a limited number of tetra- and pentapeptides under a limited number of growth conditions (reviewed by Becker and Naider, 1995). Therefore, as initial strategy the *S. cerevisiae* di-/tripeptide transport mutant PB1X-9B was transformed with a high copy number *C. albicans* genomic library and screened for the ability of *S. cerevisiae* to grow on a normally non-utilized tetrapeptide as a sole source of auxotrophic supplements.

A pRS202 based *C. albicans* genomic library was transformed into *S. cerevisiae* PB1X-9B and 32,000 *URA3*$^{+}$ transformants were obtained. Transformants were pooled into 6 groups of approximately 5,200 transformants each and subsequently plated onto a medium containing 50 μM Lys-Leu-Leu-Gly (KLLG) as the sole source of leucine and lysine as well as ammonium sulfate as a nitrogen source. A double auxotrophic selection was employed to preclude the possibility of cloning the *C. albicans LEU2* or *LYS1* homologs. *S. cerevisiae* PB1X-9B can not utilize the tetrapeptide KLLG as a sole source of lysine or leucine when grown on a medium containing a rich nitrogen source such as ammonium sulfate (unpublished observation). Oligopeptide transport (*OPT*) positive colonies appeared after 5-7 days of incubation at 30 C.

Curing of the plasmid by growth in nonselective conditions as well as shuttling the plasmid through *E. coli* and back into *S. cerevisiae* PB1X-9B demonstrated that the *OPT* activity was plasmid borne. Subsequently two different plasmids, denoted p*OPT*1 and

p*OPT*24 containing inserts of 3.8 and 4.3 kb respectively, were recovered from a representative sample of OPT^+ colonies. Initial restriction mapping demonstrated that the smaller of the two plasmids p*OPT*1 overlapped entirely with the larger plasmid p*OPT*24. Therefore, the plasmid p*OPT*1 (Fig. 1) was used in all subsequent experiments.

Southern Blot

Southern blot analysis was done to ensure that *OPT1* was derived from *C. albicans* genomic DNA and to determine if there were other homologous genes. Genomic DNA was isolated from *C. albicans* SC5314 and digested with the restriction enzymes *HincII, BamHI/PvuII,* and *PvuII/KpnI.* The resulting fragments were separated on a 1% agarose gel and Southern blotting performed as described in materials and methods. The *PvuII/KpnI* and *PvuII/BamHI* digests were each predicted to yield one band while the *HincII* digest was predicted to yield two bands. As seen in Figure 2, each digest produced their predicted bands; 617 bp and a band of >2700 bp for *HincII* (Lane 1), 790 bp for *PvuII/BamHI* (Lane 2), 1163 bp for *PvuII/KpnI* (Lane 3).

Nucleotide and Deduced Amino Acid Sequence of *OPT1*

Sequence analysis revealed the presence of two ORFs, separated by a type II intron, and encoding a hydrophobic protein of 783 amino acids with an apparent MW of 88 kD and a pI of 7.1 (Fig. 3). The first ORF contained 1626 nucleotides while the second ORF contained 723 nucleotides excluding the stop codon. The intron separating the two ORFs was 58 nucleotides in length and contained the highly conserved 5Õ splice site (GCATGT), 3Õ splice site (TAG), and branch point (TACTAAC) (Rymond and Rosbash, 1992). The two ORFs and intron constitute the gene *OPT1*. The size and

hydrophobic nature of the predicted protein product of *OPT1* are suggestive of a membrane bound protein with at least twelve putative transmembrane domains of 20-24 amino acid residues. These domains form the pathway through which the transported molecular cross the membranes.

Fragments of the nucleotide sequence of Figure 1, especially from nucleotide 1 to 2410 are within the scope of the invention providing the fragment(s) is functional to encode the oligopeptide transporter described herein or a functional part thereof. Likewise, nucleotide sequences which are adequately homologous to all or a functional part of the sequence of Figure 1 or the sequence of nucleotide 1 to 2410, are within the scope of the invention.

A search of the database using the BLAST algorithm (Althsul *et al.*, 1990) identified two ORFs from *S. cerevisiae* and one ORF from *S. pombe* as having significant homology. The ORFs SCYJL212C and YSCP9677 from *S. cerevisiae* were identified during the genome sequencing project and were not assigned any function. The remaining ORF, *ISP4* from *S. pombe*, was identified as a gene of unknown function that was up-regulated as a result of inducing meiosis through nitrogen starvation (Sato *et al.*, 1994). However, whether this induction was meiosis specific or due simply to nitrogen starvation was not determined.

The predicted protein products of the putative homologs were aligned (Fig. 4) using the PileUp program (Feng and Doolittle, 1987) from the Genetics Computer Group (GCG) software (Devereux *et al.*, 1984) and percent identity and similarity calculated using the GCG program Bestfit. The protein Isp4p from *S. pombe* exhibited the best homology with 48% identity and 70% similarity. The two proteins from *S. cerevisiae*

exhibited lower homology with 40% identity and 63% similarity for SCJL212C and 34% identity and 59% similarity for YSCP9677.

The PTR family of peptide transporters is characterized by the signature motif FYXXINXGSLS (Steiner, *et al.*, 1995) whereas the ABC transporters are characterized by the ATP binding Walker motifs (Higgins, 1992). The predicted protein product of *OPT1* did not contain the PTR signature motif or the ABC Walker motif. Furthermore, a comparison of *OPT1* with the PTR di-/tripeptide transporter *CaPTR2* using the GCG program Bestfit revealed only 18% identity between the two transporters. These data indicated that *OPT1* is not a member of the PTR or ABC families of membrane transporters.

Peptides as Growth Substrates

To determine the size constraints of peptide utilization mediated by p*OPT*1, the ability of *S. cerevisiae* PB1X-9B (a mutant in the di-/tripeptide transporter) harboring either pRS202 (the parent vector) or p*OPT*1 (pRS202 containing the 3.8 kb insert with the *OPT1* gene) to grow on KL, KLG, KLLG, and KLLLG as a sole source of leucine was tested. Previously it has been shown that di- /tripeptide transport activity in *C. albicans* and *S. cerevisiae* is regulated by nitrogen; rich nitrogen sources such as ammonium sulfate repress, while poor nitrogen sources such as proline derepress transport. Therefore simultaneous effect of nitrogen source on oligopeptide transport activity was determined by supplying either ammonium sulfate or proline as the nitrogen source. When grown on a medium containing ammonium sulfate, PB1X-9B(p*OPT*1) was only able to utilize the peptide KLLG as a source of leucine whereas PB1X-9B(pRS202) did not utilize any of the

peptides tested. When grown on a medium containing 0.1% proline, PB1X-9B(p*OPT*1) was also able to utilize the tetrapeptide KLLG as a sole source of leucine, although the growth was much more robust than the growth exhibited on the ammonium sulfate medium. No growth was observed on KL, KLG and KLLLG for PB1X-9B(p*OPT*1) or PB1X-9B(pRS202).

Sensitivity of *S. cerevisiae* Transformants to Toxic Peptides

S. cerevisiae PB1X-9B is sensitive to the toxic amino acid ethionine (Eth) but is resistant to ethionine containing di-, tri-, tetra-, and pentapeptides. Disk sensitivity assays was utilized to determine if cells transformed with p*OPT*1 were sensitive to toxic peptides and whether this sensitivity was dependent upon nitrogen source. In those conditions where ammonium sulfate was used as a nitrogen source, no zone of growth inhibition was seen for the transformed strain in the presence of AEth, LEth, or KLEth, whereas a 33 mm zone of inhibition was seen for ethionine alone (Table 1). A small and diffuse zone of growth inhibition (about 11-15 mm) was seen for KLLEth, KLAEth, and KLLAEth. When 0.1% proline was used as a nitrogen source, a zone of complete growth inhibition was seen for the toxic peptides KLLEth, KLAEth, and KLLAEth for PB1X-9B(p*OPT*1) but not for PB1X-9B(pRS202) (Fig. 5; Table 1). Neither strain exhibited sensitivity to the toxic dipeptide or tripeptide AEth and KLEth when proline was used as a nitrogen source.

The peptides can be used as a carrier for biologically active molecules, like polyoxins, nikkomycins, neopolyoxins, the latter two being peptidyl-nucleotides, which can be synthetically altered.

Transport of Lys-Leu-Gly-[^{3}H]Leu in *S. cerevisiae* Transformants

To determine if *S. cerevisiae* transformants harboring p*OPT*1 could accumulate a radiolabeled tetrapeptide, uptake assays were performed with the radiolabeled substrate KLG-[^{3}H]L with cells grown to log phase in SC-Ura with either ammonium sulfate or 0.1% proline as a nitrogen source. PB1X-9B(p*OPT*1) grown in SC with ammonium sulfate exhibited a significant uptake rate compared to no uptake by PB1X-9B(pRS202) (Fig. 6a). Furthermore, PB1X-9B(p*OPT*1) demonstrated a higher initial rate of uptake when compared to *C. albicans* SC5314 grown in the same medium. This higher initial rate can be explained by overexpression due to high copy number or alternatively by the lack of requisite regulatory elements which may be absent in the heterologous host. All three strains had a higher rate of initial uptake when grown in SC-Ura with 0.1% proline as a nitrogen source (Fig. 6b). PB1X-9B(pRS202) did accumulate the tetrapeptide KLGL under these conditions but apparently not to a large enough extent to support growth on KLLG or to exhibit sensitivity to KLLEth or KLAEth (Table 1; Fig. 5).

To more rigorously determine the size constraints of the oligopeptide transporter, the accumulation of KLG-[^{3}H]L was measured in the presence of 10-fold molar excess of the competitors L, KL, KLG, KLLG, and KLLLG. SC-Ura with ammonium sulfate was chosen as the growth medium because under these growth conditions PB1X-9B(p*OPT*1) accumulated KLG-[^{3}H]L whereas PB1X-9B(pRS202) did not (Figure 6a). As seen in Figure 7, L and KL do not compete with the uptake of KLG-[^{3}H]L whereas competition was seen with KLLG and KLLLG. The tripeptide KLG exhibited decreased competition in comparison to KLLG or KLLLG (Fig. 7) possibly due to a lower affinity, although this low level of KLG uptake is below the threshold to support full growth when used as an auxotrophic supplement. Uptake rates were calculated from a bestfit of the slope for each set of data. The uptake rate of KLG-[^{3}H]L in the presence of no competitor, L, or KL

was 0.24, 0.25, and 0.26 nm/min/mg of dry weight, respectively. When KLG was used as a competitor the uptake rate was 0.12 nmoles/min/mg of dry weight which was approximately 50% of the no-competitor rate. The uptake rate approximated zero when KLLG and KLLLG were used as competitors.

DETAILED DISCUSSION OF THE PREFERRED EMBODIMENT

Characterization of the oligopeptide transport gene proceeded as follows. Three lines of evidence support the cloning of an oligopeptide transport gene from *C. albicans*. First, the plasmid p*OPT*1 conferred the ability to utilize the peptide KLLG to satisfy the leucine auxotrophic requirement of *S. cerevisiae* PB1X-9B when grown on a medium with a rich or poor nitrogen source. Secondly, the *S. cerevisiae* strain PB1X-9B was not sensitive to the toxic peptides KLLEth, KLAEth, or KLLAEth when grown on a minimal medium with 0.1% proline as a nitrogen source but was sensitive when transformed with the plasmid p*OPT*1 (Fig. 5; Table 1). Similarly, a very faint zone of growth inhibition was seen for KLLEth, KLAEth, and KLLAEth when PB1X-9B(p*OPT*1) but not PB1X-9B(pRS202) was grown in a medium containing ammonium sulfate. Finally, PB1X-9B(pRS202) had an initial uptake rate of zero for the radiolabelled substrate KLG-[^{3}H]L when grown on a media with ammonium sulfate whereas PB1X-9B(p*OPT*1) had a dramatically higher initial uptake rate (Fig. 6a). When the growth media contained proline as a nitrogen source, the initial uptake rate was 2.5 times higher for PB1X-9B(p*OPT*1) than for PB1X-9B(pRS202) (Fig. 6b). Furthermore, uptake was competed by KLLG and KLLLG and to a lesser degree KLG (Figure 7). The fact that leucine did not compete with KLG-[^{3}H]L for uptake excluded the possibility that *OPT1* coded for a

secreted protease. Therefore, these studies demonstrated the cloning of an oligopeptide transporter from *C. albicans* capable of transporting tetra- and pentapeptides and to a lesser extent tripeptides.

Characterization of the protein product proceeded as follows. The predicted protein product of *OPT1* did not show any significant homlogy to any members of the ABC superfamily or PTR family of transporters. Furthermore, a search of the Prosite (Bairoch, 1992) and Motifs (Devereux *et al.*, 1984) databases for protein motifs did not reveal any previously identified functional domains common to transport proteins with the exception of potential glycosylation sites. However, are the twelve putative transmembrane domains separated by hydrophilic regions as well as the expression of transport activity in a heterologous host are suggestive of an integral membrane transporter.

Because three ORFs of significant homology as well as several Expressed Sequence Tags (data not shown) were identified, the possibility exists that *OPT1* constitutes the first identified member of a new family of transporters. It is not excluded that one or more of these ORFs may encode proteins that have oligopeptide transport activity. Thus, one or more of these domains could be expressed from appropriate nucleotide sequences and retain a transporter function.

It was found that Opt1p is able to accommodate peptides of 3-5 residues. It is not excluded that oligopeptide activity would include peptides longer than pentapeptides. The protein encoded by *OPT*1 is isolated in accordance with known protocols. Asubel *et al.*, Current Protocols. As demonstrated by growth assays, halo assays, and competition experiments tetrapeptides were most readily transported by Opt1p. On the other hand, pentapeptides did enter the cell as demonstrated by sensitivity to KLLAEth and supported

by the competition between KLLLG and KLG-[^{3}H]L. However, KLLLG was not able to support growth when used as a source of leucine possibly due to the inability of cellular peptidases to release leucine from this peptide. Similarly, KLG was able to compete slightly for entry into the cell with KLG-[^{3}H]L, but KLG did not support growth and KLEth was not toxic. From these studies it can not be concluded that Opt1p has a lower affinity for tri- and pentapeptides than for tetrapeptides.

Nucleotide sequence analysis revealed the presence of a 58 nucleotide intron located within the 3Õ half of *OPT1*. The 5Õ splice site, 3Õ splice site, and branch point are identical to previously reported type II introns within fungi (Rymond and Rosbash, 1992). It is interesting to note that the di-/tripeptide transporter *CaPTR2* also contains a small type II intron that is located within the 3Õ half of the gene. It has been suggested that introns play a regulatory role. However, a comparison of the two introns did not reveal any apparent consensus sequences that might be suggestive of a common regulatory element or of a common ancestory.

To date only one study has been published addressing the regulation of oligopeptide transport activity in *C. albicans*. Basrai *et al.*, (1992) concluded that sensitivity to toxic oxalysine-containing tetra- and pentapeptides was not influenced by nitrogen source or by the presence of amino acid inducers. However, our findings suggest that when expressed in *S. cerevisiae, OPT1* is regulated by nitrogen source. The discrepancy in results may be explained by differences in the levels of regulation or substrate specificity between the two different strains used in the studies , or alternatively by superimposition of a *S. cerevisiae* regulatory mechanism on the *CaOPT1* gene expressed heterologously.

A search of the database using the BLAST algorithm identified three putative homologs of *OPT1*. The *ISP4* gene from *S. pombe* exhibited the highest homology and

was identified by Sato *et al.* (1994) through a subtractive hybridization experiment using RNA isolated from nitrogen starved and non-nitrogen starved cells. In *S. pombe*, nitrogen starvation induces meiosis and therefore this nitrogen-starvation/meiosis-inducing screen identified genes that were either induced during meiosis or regulated by the nitrogen catabolite repression system. Based upon the high homology between *OPT1* and *ISP4* and the established role of nitrogen regulation in many peptide transport systems, it was hypothesized that *ISP4* encodes an oligopeptide transporter that is regulated by nitrogen source.

The remaining two putative homologs were from *S. cerevisiae* and were identified during the genome sequencing project. Interestingly, few favorable conditions have been identified for oligopeptide transport activity in *S. cerevisiae*. As seen in Figure 6, PB1X-9B(pRS202) when grown in a medium containing proline exhibited an initial uptake rate of KLG-[^{3}H]L that was comparable to the initial uptake rate of PB1X-9B(p*OPT*1) when grown in a medium with ammonium sulfate. However, under these conditions PB1X-9B(pRS202) exhibited no sensitivity to the toxic tetrapeptides KLLEth and KLAEth and was not able to utilize the tetrapeptide KLLG as a sole source of leucine, whereas PB1X-9B(p*OPT*1) did grow on KLLG and exhibited slight sensitivity to the toxic peptides KLLEth and KLAEth. This discrepancy in results could be explained by an uptake rate exhibited by PB1X-9B(pRS202) that may not necessarily be reflective of total peptide accumulation over the prolonged incubation times necessary for growth and sensitivity assays.

MATERIALS AND METHODS

Strains, Vectors and Media

The strains used herein were *S. cerevisiae* PB1X-9B (*MATa ura3-52 leu2-3,112 lys1-1 his4-38 ptr2-2*) (Perry *et al.*, 1994) and *C. albicans* SC5314 (Fonzi and Irwin, 1993).

C. albicans and *S. cerevisiae* cells were maintained on YEPD medium (2% dextrose, 1% Yeast Extract, 2% Peptone, and 1.5% agar). The minimal medium used for most studies was made by adding 10 ml of 10X filter sterilized YNB (Yeast Nitrogen Base, Difco) with ammonium sulfate and without amino acids to 90 ml of sterile water containing 2 g glucose and auxotrophic supplements (Sherman *et al,* 1986). For those experiments where proline was used as a sole nitrogen source YNB without amino acids and without ammonium sulfate was supplemented with 0.1% proline. The mutant strain *S. cerevisiae* PB1X-9B was grown in Synthetic Complete Medium (SC) which consisted of minimal medium with histidine, uracil, lysine and leucine. *S. cerevisiae* PB1X-9B transformed with pRS202 based plasmids was grown on SC lacking uracil (SC-Ura).

The *C. albicans* library used for cloning *OPT1* was provided by Gerry Fink (Liu *et al.*, 1994). The library was created by partially digesting *C. albicans* strain 1006 genomic DNA (Goshorn and Sherer, 1989) with *Sau3A* and cloning the resulting fragments (>4 kb) into the *SalI* site of pRS202, a *URA3/2 μ* based plasmid (Christianson *et al.*, 1992).

Peptide medium consisted of minimal medium supplemented with auxotrophic requirements minus the amino acid leucine plus 100 μM of one of the following peptides: Lysyl-Leucine (KL), Lysyl-Leucyl-Glycine (KLG), Lysyl-Leucyl-Leucyl-Glycine (KLLG), Lysyl-Leucyl-Glycyl-Leucine (KLGL), or Lysyl-Leucyl-Leucyl-Leucyl-Glycine (KLLLG). Abbreviations for toxic peptides and amino acids used herein are as follows:

Ethionine (Eth), Alanyl-Ethionine (AEth), Leucyl-Ethionine (LEth), Lysysl-Leucyl-Ethionine (KLEth), Lysyl-Leucyl-Leucyl-Ethionine (KLLEth), Lysysl-Leucyl-Alanyl-Ethionine (KLAEth), and Lysyl-Leucyl-Leucyl-Leucyl-Ethionine (KLLLEth). All amino acids were in the *L* configuration.

Enzymes, chemicals and reagents

Restriction endonucleases, T4 DNA ligase, T4 DNA polymerase, and alkaline phosphatase were purchased from New England BioLabs or Promega and were used according to the manufacturers specifications.

Synthesis of Radioactive Lys-Leu-Gly-[^{3}H]Leu

KLGL was prepared by conventional automated solid phase peptide synthesis on an Applied Biosystems Model 433A synthesizer. Peptide was cleaved from the resin with trifluoroacetic acid (TFA) and purified using a C_{18} reversed phase column (19 x 300 mm) to >99% homogenity with a 5 to 20% linear gradient of acetonitrile in water over 60 minutes. The product was verified using mass spectrometry [$(M^+ + 1) = 430.2$; calculated$=429.6$].

Tritiated KLGL (Lys-Leu-Gly-[^{3}H]Leu) was prepared by solution phase peptide synthesis as follows. BocLys(Boc)-Leu-Gly-OH (5.2 mg; 10 μmol) was dissolved in 108 μl of a 0.092 μmol/μl solution of N-hydroxysuccinimide (10 μmol) in dry dioxane. Dicyclohexylcarbodiimide (10 μmol in 57 μl) in dry dioxane was added and the reaction mixture was stirred for one hour at ambient temperature. Leu (0.65 mg, 5 μmol),

dissolved in 1 ml of water, was added to radioactive leucine (American Radiolabeled Chemicals, Inc., St. Louis, MO; Specific Activity 60 Ci/mmol; Concentration 1mCi/ml in 2% ethanol). This solution was evaporated to dryness, redissolved in 250 μl of water/dioxane (4:1) containing N-methyl morpholine (50 μmol) and the solution containing the activiated tripeptide was added. The resulting reaction mixture was stirred for 6 hours at ambient temperature, 5.5 ml of TFA was then added, and after 5 minutes the reaction mixture was evaporated to dryness. The residue was redissolved in 500 μl of water, injected onto a Waters μBondapack C_{18} column (7.8 x 300 mm) and eluted isocratically using 5% acetonitrile in water, containing 0.025% TFA. Product elluting at the KLGL position was collected, evaporated, redissolved in water (200 μl) and analyzed by high pressure liquid chromatography (HPLC) and on silica thin layers using a Butanol:Acetic acid:water (4:1:5) mobile phase. TLC plates were exposed to film overnight at -80 C and developed to show one radioactive spot with the mobility of the desired tetrapeptide. KLG-[^{3}H]L was >97% pure according to HPLC. Specific radioactivity was 90 mCi/mmol. Peptide was diluted with nonradioactive KLGL as required.

DNA Manipulations

Small scale plasmid DNA preparations from *E. coli* transformants were performed as described in Sambrook *et al.* (1989). Plasmid DNA from *S. cerevisiae* transformants was isolated as described previously (Ward, 1990). Whole cell DNA from *C. albicans* was obtained by the procedure described by Ausubel *et al.*(1990).

Yeast transformations were done using the procedure described by Gietz *et al.* (1991) and plates were incubated at 30 ¡C for 4 days or longer.

For Southern analyses whole cell DNA was digested with restriction enzymes and electrophoresed on 1.0% agarose gels. Southern blotting was done as described in Sambrook *et al.* (1989). Hybridization was performed at 60° C for twelve hours in a Hybritube (Gibco BRL) followed by two washes of 1X SSC, 0.1% SDS at 42° C and two washes of 0.1X SSC and 0.1% SDS at 60° C. The probe used for Southern blots was generated via PCR using the primers LC2 (5Õ GCATGGATTGTTCCTGACTGG 3Õ) and FT2 (5Õ CCAATACCAAACAAATGAGGC). The product was 408 bp in length and its position within the *OPT1* ORF is depicted in Figure 1. The Southern blot displayed in Figure 2 was processed using the program Adobe Photoshop.

For plasmid curing experiments *S. cerevisiae* transformants were grown nonselectively in YEPD broth for about 40 generations. Cells were then plated on YEPD plates to obtain isolated colonies which were picked, washed with water, resuspended at 5×10^6 cells/ml in sterile water, and spotted onto the appropriate peptide medium.

The nucleotide sequence of the 3.8 kb insert in plasmid p*OPT*1 was generated through automated cycle sequencing using an ABI 373A Automated sequencer (Smith *et al.*, 1986). The insert of p*OPT*1 was digested with either *TaqI* or *Sau3A*, subcloned into M13, and ssDNA isolated as a template for sequencing from randomly chosen plaques. The sequenced fragments were assembled using the software DNASTAR and the remaining gaps were filled using properly placed primers. Primers were purchased from Bioserve Biotechnologies. Final assembly was performed using Autoassembler from ABI.

Growth and transport assays

Growth assays to determine the phenotype of the cells were done as described by Island *et al.* (1991). Briefly, 3 μl of culture from a suspension of 5 x 10^6 cells/ml were spotted to the surface of the medium and plates were incubated at 30 ¡C for 4-7 days.

Uptake of KLG-[^{3}H]L was determined using a protocol for uptake of dipeptides as described by Basrai *et al.* (1995) with a few modifications. *S. cerevisiae* cultures were grown overnight to exponential phase in SC-Ura medium. Cells were harvested by centrifugation, and resuspended in 2% glucose at a cell density of 2 X10^8 cells/ml. Two hundred and fifty microliters of cell suspension were added to an equal volume of an uptake assay reaction mixture and incubated at 30¡C. The final concentrations of the components in the uptake assay solution were: glucose (2%, w/v), 10mM sodium citrate/potassium phosphate buffer (pH 5.0), and KLG-[^{3}H]L (150 μM; 8.5 mCi/mmol). Competition experiments were done in the presence of either 1.5 mM L, KL, KLG, KLLG, or KLLLG. At various time points, 90 μl portions were removed and filtered through a membrane. The yeast cells retained on the filter were washed twice with ice-cold distilled water, once with room temperature distilled water, and the residual radioactivity was measured by liquid scintillation. There was no peptide adsorption to the cell surface or sticking to filters since at 0¡ C the counts were at background level. The uptake results, calculated on the basis of 50% counting efficiency (determined using L-[^{3}H]lysine as a standard, and the specific activity of the peptide), were expressed as nmol of peptide uptake per minute per mg cell dry weight.

Sensitivity assays

Sensitivity to ethionine, a toxic methionine analog, and ethionine-containing peptides was determined by the method described by Island *et al.* (1987). Cells were

grown overnight to exponential phase of growth in SC, washed, and resuspended at 5 x 10 6 cells /ml in sterile water. One ml of this cell suspension was added to 3 ml molten Noble agar (0.8% final concentration) and overlayed on 20 ml of the same medium used to prepare the inoculum. A disk (6 mm diameter, Difco) was placed on the plate and 0.38 µmoles of the compounds to be tested were applied to the disks. Zones of inhibition were measured after 24-48 hr incubation at 30 ¡C. Each test comprised at least three independent assays and the results represented in the Tables are means of the values obtained. Maximum variation between the zones of inhibition measured for each test were ²3 mm. A value of 7 mm for the diameter of zone of inhibition represents a minimal growth inhibition value as the disk diameter was 6 mm. Photodocumentation of sensitivity assays was done with a Umax Scanner and processed through Adobe Photoshop.

REFERENCES

All references are incorporated herein by reference in their entirety.

Altshul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *J. Mol. Biol.* **215**:403-410.

Ausubel, F. M., Brent, R., Kingston, R., Moore, D. D., Seidman, J. G.,. Smith, J. A., & Struhl, K. (1990). *Current Protocols in Molecular Biology* **2**. New York: Wiley Interscience, Inc.

Bairoch. (1992). PROSITE: a dictionary of sites and patterns in proteins. *Nucl. Acids Res.* **20**: 2013-2018.

Basrai, M.A., Lubkowitz, M. A., Perry, J. R., Miller, D., Krainer, E., Naider, F., & Becker, J. M. (1995). Cloning of a *Candida albicans* peptide transport gene. *Microbiology UK* **141:** 1147-1156.

Basrai, M. A., Zhang, H-L., Miller, D., Naider, F., & Becker, J. M. (1992). Toxicity of oxalysine and oxalysine-containing peptides against *Candida albicans*: regulation of peptide transport by amino acids. *J. Gen. Microbiol.* **138:**2353-2362.

Becker, J. M. & Naider, F. (1995). Fungal peptide transport as a drug delivery system. In *Peptide Based Drug Design: Controlling Transport and Metabolism*, pp. 369-384. Edited by M. Taylor and G. Amidon. Washington, DC: American Chemical Society.

Christianson, T. W., Sikorski, R. S., Dante, M., Shero, J. H., & Heiter, P. (1992). Multifunctional yeast high copy number shuttle vectors. *Gene* **110:** 119.

Cundell, D.R., Pearce, B.J., Sandros, J., Naughton, A. M., & Masure, H. R. (1995). Peptide permeases from *Streptococcus pneumoniae* affect adherence to eukaryotic cells. *Infection and Imunnity* **63:** 2493-2498.

Devereux, Haerbili, J. P., & Smithies, O. (1984). A comprehensive set of sequence analysis programs for the VAX. *Nucl. Acids Res.* **12:**387-395.

Feng, D.-F., & Doolittle, R. F. (1987). Progressive sequence alignment as a prerequisite to correct phylogenetic trees. *J. Mol. Evol.* **25:**351-360.

Fonzi, W. A. & Irwin, M. Y. (1993). Isogenic strain construction and gene mapping in *Candida albicans*. *Genetics* **134:**717-728.

Gietz, D., Andrew, J., Woods, R., & Schiestl, R. (1991). Improved methods for high efficiency transformation of intact yeast cells. *Nucl. Acids Res.* **20:**1425.

Goshorn, A. G., & Sherer, S. (1989). Genetic analysis of prototrophic natural variants of *Candida albicans*. *Genetics* **123:** 667-673.

Hagting, A., Kunji, E. R., Leenhouts, K. J., Poolman, B., & Konings, W. N. (1994). *J. Biol. Chem.* **269:** 11391-11399.

Higgins, C. F. (1992). ABC transporters: from microorganisms to man. *Annu. Rev. Cell Biol.* **8**:67-113.

Island, M. D., Naider, F., & Becker, J. M. (1987). Regulation of dipeptide transport in *Saccharomyces cerevisiae* by micromolar amino acid concentrations. *J. Bacteriol.* **169:**2132-2136.

Island, M. D., Perry, J. R., Naider, F., & Becker, J. M. (1991). Isolation and characterization of *S. cerevisiae*Êmutants deficient in amino acid-inducible peptide transport. *Curr. Genetics* **20:**457-463.

Liu, H., Kohler, J., & Fink, G. (1994). Suppression of hyphal formation in *Candida albicans* by mutation of a *STE12* homolog. *Science* **266:** 1723-1725.

McCarthy, J. P., L. J. Nisbet, J. C. Boehm, & Kingsbury, W. D. (1985). Multiplicity of peptide permeases in *Candida albicans* : Evidence from novel chromophoric peptides. *J. Bacteriol.* **162:**1024-1029.

Milewski, S., Andruskiewicz, R., & Borowski, E. (1988). Substrate specificity of peptide permeases in *Candida albicans*. *FEMS Microbiol.Letters* **50:**73-78.

Omaha, T., Suzuki, T., Mori, M., Osawa, S., Ueda, T., Watanabe, K., & Nakase, T. (1993). Non-universal decoding of the leucine codon CUG in several *Candida* species. *Nucl. Acids. Res.* **21:** 4039-4045.

Parra-Lopez, C., Baer, M. T., & Groisman, E. A. (1993). Molecular genetic analysis of a locus reqquired for resistance to antimicrobial peptides in *Salmonella typhimurium*. *EMBO.* **12:** 4053-4062.

Payne, J. W. & Shallow, D. A. (1985). Studies on drug targeting in the pathogenic fungus *Candida albican:* peptide transport mutants resistant to polyoxins, nikkomycins and bacilysin. *FEMS Micriobiol. Letters* **28:** 55-60.

Payne, J. W. & Smith, M. W. (1994). Peptide transport by micro-organisms. *Adv. Microbiol. Phys.* **36:** 1-80.

Perry, J. R., Basrai, M. A., Steiner, H-Y., Naider, F., & Becker, J. M. (1994). Isolation and Characterization of a *Saccharomyces cerevisiae* Peptide Transport Gene. *Mol. Cell. Biol.* **14**: 104-115.

Rymond, B.C., & Rosbash, M. (1992). Yeast pre-mRNA splicing. p.143-192. *in* E. W. Jones, J.R. Pringle, and J. R. Broach (ed.), *The Molecular and Cellular Biology of the Yeast Saccharomyces.* Cold Spring Harbor Press, Cold Spring Harbor, New York.

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). *Molecular Cloning, A Laboratory Manual.* Second Edition. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Sato, S., Suzuki, H., Widyastuti, U., Hotta, Y., & Tabata, S. (1994). Identification and characterization of genes induced during sexual differentiation in *Schizosaccharomyces pombe*. *Current Genetics* **26:** 31-37.

Sherman, F., Fink, G. R., & Hicks, J. B. (1986). In *Methods of Yeast Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Smith, L. M., Sander, J. Z., Kaiser, R. J., Hughes, P., Dodd, C., Connel, C. R., Heiner, C., Kent, S. B., & Hood, L. E. (1986). Flourescence detection in automated DNA sequence analysis. *Nature.* **321:674-679.**

Steiner, H-Y., Naider, F., & Becker, J. M. (1995). The PTR family: a new group of peptide transporters. *Mol. Microbiol.* **16:** 825-834.

Steiner, H-Y., Song, W., Naider, F., Zhang, L., Stacey, G., & Becker, J. M. (1994). An *Arabidopsis* peptide transporter is a member of a new class of membrane transport proteins. *Plant Cell* **6:** 1289-1299.

Song,W., Steiner, H-Y., Zhang, L., Naider, F., Stacey, G., & Becker, J. M. (1996). Cloning of a second *Arabidopsis* peptide transport gene. *Plant Physiol.* **110:** 171-178.

Ward, A. C. (1990). Single step purification of shuttle vectors from yeast for high frequency back-transformation into *E. coli*. *Nucl. Acids Res.* **18:** 5319.

Yadan, J. C., Gonneau, M., Sarthou, P., & Le Goffic, F. (1984). Sensitivity to

nikkomycin Z in *Candida albicans*: Role of peptide permeases. *J. of Bacteriol.* **160:** 884-888.

CLAIMS

What is claimed is:

1. An oligopeptide membrane transporter which is not a member of the PTR or ABC families.

2. The oligopeptide membrane transporter of claim 1 which is from *Candida albicans*.

3. The oligopeptide membrane transporter of claim 1 which is competent to transport tetra- and pentapeptides.

4. The oligopeptide membrane transporter of claim 3 which is competent to transport the peptides in a heterologous host.

5. The oligopeptide membrane transporter of claim 4 wherein the peptides are toxic peptides.

6. The oligopeptide membrane transporter of claim 1 which is encoded by a nucleic acid sequence which comprises the sequence of Figure 1.

7. The oligopeptide membrane transporter of claim 6 which is encoded by a nucleotide sequence of Figure 1 comprising 2407 nucleotides.

8. An isolated nucleotide sequence which comprises the nucleotide sequences of Figure 1.

9. The isolated nucleotide sequence of claim 8 wherein the nucleotide sequence comprises 2407 nucleotides.

10. The isolated nucleotide sequence of claim 9 wherein the nucleotide sequence comprises two ORFs, a first comprising 1626 nucleotides and a second comprising 723 nucleotides.

11. The isolated nucleotide sequence of claim 10 wherein the two ORFs are separated by an intron comprising 58 nucleotides.

12. The isolated nucleotide sequence of claim 11 which is a gene encoding an oligopeptide membrane transporter.

13. The isolated nucleotide sequence of claim 12 wherein the transporter is competent to transport tetra- and pentapeptides in a heterologous host.

14. The isolated nucleotide sequence of claim 13 wherein the peptides are toxic peptides.

15. An isolated nucleotide sequence which encodes an oligopeptide membrane transporter which is not a member of the PTR or ABC families.

16. The nucleotide sequence of claim 15 wherein the transporter is competent to transport tetra- or pentapeptides in a heterologous host.

17. The nucleotide sequence of claim 16 wherein the peptides that are transported are toxic peptides.

18. A eukaryotic host transformed with a nucleic acid sequence which encodes an oligopeptide membrane transporter competent to transport tetra- and pentapeptides.

19. The host of claim 10 which is a *Saccharomyces*.

20. The host of claim 11 which is *S. cerevisiae*.

21. The host of claim 19 wherein the nucleotide sequence is from *C. albicans*.

22. A method for transporting an oligopeptide through a cell lipid membrane which comprises causing a *Saccharomyces* yeast transformed with a nucleotide sequence comprising the sequence of 1 to 2407 nucleotides shown in Figure 1 to utilize a tetra- or pentapeptide, causing it to traverse the cells'.lipid membrane mediated by the peptide transporter encoded by said nucleotide sequence and delivering the oligopeptide to the target.

23. The method of claim 23 wherein the nucleotide sequence is the gene encoding the oligopeptide membrane transporter.

24. The method of claim 23 wherein the peptide is a carrier for a toxic molecule for delivery to the target.

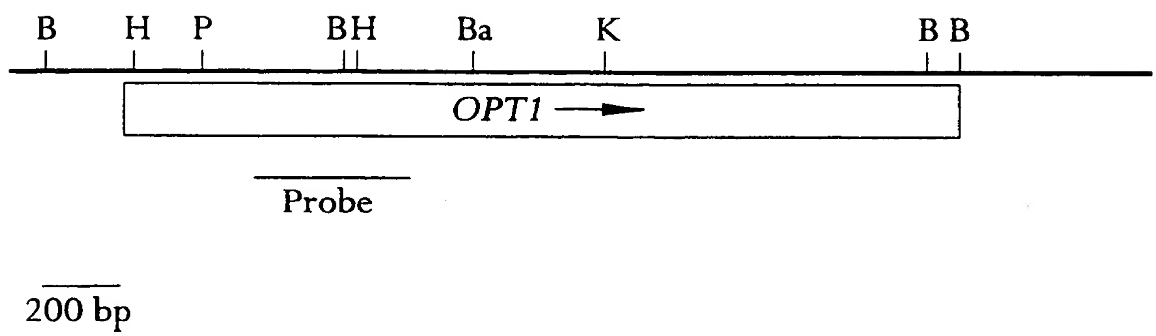

FIG. 1

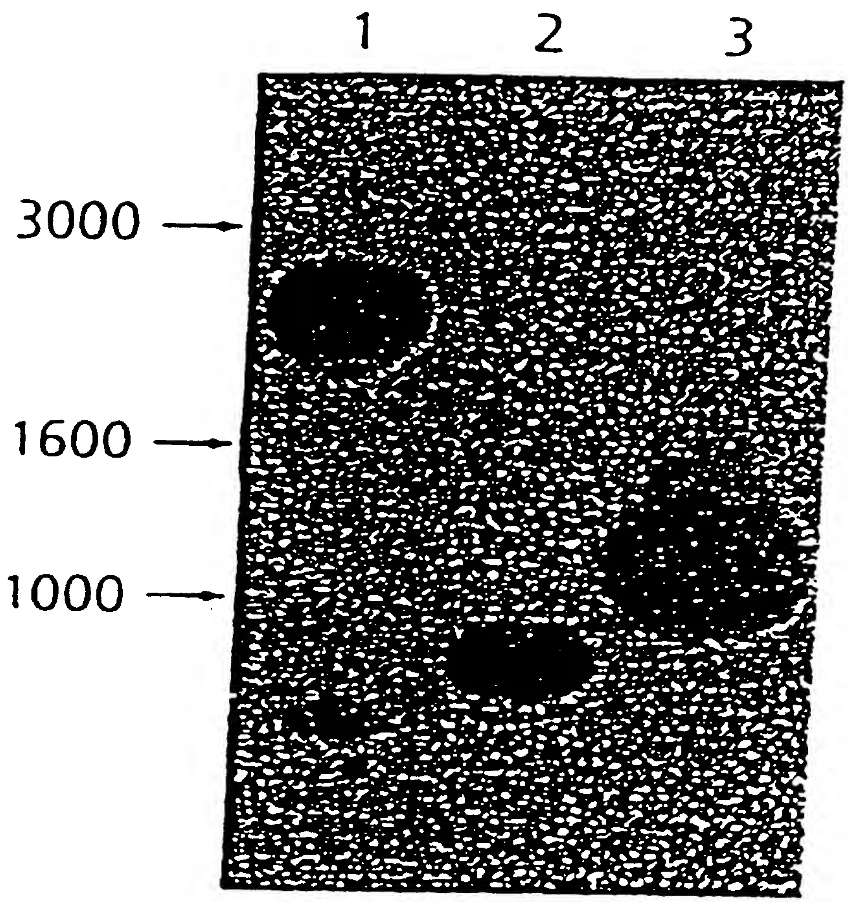

FIG. 2

```
                                 GATCATGTGGCTAAAAATTCATATGGCTTTATGCTGTGGCTTCAGCTCTACTGATAATTGATATCTTTATT  -410
     CCTATACATAAATATTAAAGCCACTTGATTATTGCTCATAGGGCCAAAAAAACAAAAAGATGCAGAACCATCTAAAGTTTTGTTTTGTGTTTGCTATT  -312
     TTGTGCCTAGTGAGATTAAATTAGTTATCTTTTCATGACAAAAATCCTTTAGACTACTTTTTATTCCATTTGTTTGGTTTACGATTATCAATCGTCAT  -214
     AGTTCAATTTGTAAAATTTTATCTTTTCAATCCCAAACCTTTTAAATAGTCAGTAATTTCTCTCATAGGAATTTCAAGTTTCCACTTTTTTTTGTCTT  -116
     CCTTCTATTTTCTTTTTATAAGTTTACTGTTTCGTGAAATATTATTCATTTGTATTATTTTTACTAAGTCAACCACTATTGATTCCATTCCTAACACT  -18
     TATTATAAGTACTTACT                                                                                     -1

     ATG GAC AAA ATA AGG GCA GTA ATT AGT GGA GGT GAG AAA CCT CCC GTT GAC ACT GAC AAC GAT CAC AAC ACA GAC   75
1    M   D   K   I   R   A   V   I   S   G   G   E   K   P   P   V   D   T   D   N   D   H   N   T   D
     TTT GAG GCT GAC AGA AAA ATG CCA GAT TTG GAT ATT GTA GTT TCC AAA TCA CAA GAA TTT GAC CAA GTC ACC TCC  150
26   F   E   A   D   R   K   M   P   D   L   D   I   V   V   S   K   S   Q   E   F   D   P   V   T   S
     CAC TTG GTT AAT GAT ATT ATG GAA GAT GAA TAT GCT GCT GTC CAT GTT GAA GAT GAT TCT CCT TAT CCA GAA GTT  225
51   H   L   V   N   D   I   M   E   D   E   Y   A   A   V   H   V   E   D   D   S   P   Y   P   E   V
     AGA GCA GCT GTT CCT TCT ACT GAC GAC CCA ACT TTA CCT CAA AAT ACC ATT AGA GCC TGG GTT ATT GGT TTG ATA  300
76   R   A   A   V   P   S   T   D   D   P   T   L   P   Q   N   T   I   R   A   W   V   I   G   L   I
     TTG ACT ACG GTT GGT TGT GGT ATG AAT ATG TTG TTC AGT TTC CAT AGT CCC TCA TTT GCT ATC ACC ACT TTT GTC  375
101  L   T   T   V   G   C   G   M   N   M   L   F   S   F   H   S   P   S   F   A   I   T   T   F   V
     ACA TCC ATT TTG GCT TGG CCA ATT GGG AAC TTT TGG GCA TGG ATT GTT CCT GAC TGG AAG ATT TTT GGT GCT TCG  450
126  T   S   I   L   A   W   P   I   G   N   F   W   A   W   I   V   P   D   W   K   I   F   G   A   S
     TTA ATT CCA GGT CCA TTC AAC GTT AAA GAA CAT ACT ATC ATC ACT ATT ATG GCC AAC GTT TCT TTT GGT ACT GGT  525
151  L   N   P   G   P   F   N   V   K   E   H   T   I   I   T   I   M   A   N   V   S   F   G   T   G
     GCC GCA TAT GCC ACA GAT ATC TTG CTT GCA CAA AAT ATG TTT TAT AAA TCA AAT TTT GGT TGG GGG TAC AAT TTA  600
176  A   A   Y   A   T   D   I   L   L   A   Q   N   M   F   Y   K   S   N   F   G   W   G   Y   N   L
     TTA CTT ATC TGG AGT ACC CAA TGT ATT GGG TTT GCT TTC GGA GCT GTT ATG AGA AGA TTT GTT GTT GAC AGT CCA  675
201  L   L   I   W   S   T   Q   C   I   G   F   A   F   G   G   V   M   R   R   F   V   V   D   S   P
```

FIG. 3

FIG. 3a
FIG. 3b
FIG. 3c

FIG. 3a

```
    GGC GCC ATC TGG CCC CTG AAT TTG GTC ACC GCA ACA TTC TTG ACT AAT ATG CAC ATT AAC GAA AAC CAC ACT GCT  750
226 G   A   I   W   P   S   N   L   V   T   A   T   F   L   T   N   M   H   I   N   E   N   H   T   A
    AAT GGC TGG AAA ATT TCT CGT CTT GCA TTT TTC GTG ATC GTG TTT GTT GCC TCA TTT GTT TGG TAT TGG TTC CCA  825
251 N   G   W   K   I   S   R   L   A   F   F   V   I   V   F   V   A   S   F   V   W   Y   W   F   P
    GGT TAT ATT TTC CAG GCT TTA TCG TAT TTT TCT TGG ATC ACC TGG ATT AAA CCA AAC ATT GTC ATT ATC AAT CAA  900
276 G   Y   I   F   Q   A   L   S   Y   F   S   W   I   T   W   I   K   P   N   N   V   I   I   N   Q
    GTT TTC GGT TCT TCA TCT GGG TTA GGT ATG ATT CCT AAC AAC ATT GCC TTG GAC TGG AAC CAA ATT GCA GGG TAT  975
301 V   F   G   S   S   S   G   L   G   M   I   P   N   N   I   A   L   D   W   N   Q   I   A   G   Y
    ATT GGG TCT CCA TTG ATT CCA CCA GCT AGT GTT ATT GCT ACA ATT TTT GGA TCC ATT GTG CTT ATT TTC TGG ATT 1050
326 I   G   S   P   L   I   P   P   A   S   V   I   A   T   I   F   G   S   I   V   L   I   F   W   I
    GTT GTG CCA GCT ATT CAC TAT TCC AAC ACT TGG TAC TCC CAA TAC TTG CCA ATC TCA TCT ACT GGA TCG TTT GAT 1125
351 V   V   P   A   I   H   Y   S   N   T   W   Y   S   Q   Y   L   P   I   S   S   T   G   S   F   D
    AGG TTC CAA CAA ACT TAT AAT GTG TCA AAA ATT ATC GAC CAT AAA ACT TTA TCA TTC AAT GAA GCG GAA TAC AAA 1200
376 R   F   Q   Q   T   Y   N   V   S   K   I   I   D   H   K   T   L   S   F   N   E   A   E   Y   K
    AAG TAC TTC CCT TTG TTT TTA TCC ACC ACC TTT GCC ATT TCC TAT GGG CTA TCA TTT GCC TCC ATT TTA GCC ACT 1275
401 K   Y   S   P   L   F   L   S   T   T   F   A   I   S   Y   G   L   S   F   A   S   I   L   A   T
    ATA ACA CAC ACC ATT TGC TTC CAT GGA CGT GAG CTT ATC GCA TCG TTG AAG GCC AAA GAA AAA CAA GAT GTT CAC 1350
426 I   T   H   T   I   C   F   H   G   R   D   L   I   A   S   L   K   A   K   E   K   P   D   V   H
    AAT AGA TTA ATG AAA GCA TAC AAA CCA GTG CCT GAA TGG TGG TAC CTA GTT GTC TCC TTG GTC TTT TTC GTT ATG 1425
451 N   R   L   M   K   A   Y   K   P   V   P   E   W   W   Y   L   V   V   F   L   V   F   F   G   M
    TCC ATA GCC ACC GTA CGT GCT TGG CCT ACT GAA ATG CCA GTA TGG GGG TTA GTT TTT GCT CTT ATC ATC GCT ATC 1500
476 S   I   A   T   V   R   A   W   P   T   E   M   P   V   W   G   L   V   F   A   L   I   I   A   I
    ATA TTT TTA TTA CCC GTT GCT ATC ATT TAT GCA AAA ACG AAT AAT GCT GTT GGT TTA AAC GTT GTA ACC GAG TTC 1575
501 I   F   L   L   P   V   A   I   I   Y   A   K   T   N   I   A   V   G   L   N   V   V   T   E   F
    ATC GTG GGC TAC GTA CTA GGT GGA CGT CCC CTA TGT ATG ATG TTG TTC AAG|GCATGT|ATTAGAATTTGCAGATCATAATCA 1656
526 I   V   G   Y   V   L   G   G   R   P   L   C   M   M   L   F   K
    GTCAGTT|TACTAAC|CTAATTTTGAA|TAG| ACC TTC GGA TAC ATC ACT AAT AAC CAA GCT GTT ACT TTT GTG CAG GAT ATG 1735
543                                  T   F   G   Y   I   T   N   N   Q   A   V   T   F   V   Q   D   M
```

FIG. 3b

```
    AAA CTT GGG CAC TAC ATG AAA ATA GAT CCG CGC ACT TTG TTT TGG GCG CAG TTT GCT GCT ACC ATA TGG GGA TCG  1810
560 K   L   G   H   Y   M   K   I   D   P   R   T   L   F   W   A   Q   F   A   A   T   I   W   G   S
    TTA GTT CAG ATC GCA GTT TTG GAG TGG GCC TAT GGT GCA ATC GAC AAT TTG TGT GCT GCT GAC CAA AAA AAT CAT  1885
585 L   V   Q   I   A   V   L   E   W   A   Y   G   A   I   D   N   L   C   A   A   D   Q   K   N   H
    TAC ACA TGT CCA AAC GTT AAA GTT TTC TTC AAT GCT TCG ATC ATT TGG GGT GTC ATT GGA CCC CAA CGT CAA TTC  1960
610 Y   T   C   P   N   G   K   V   F   F   N   A   S   I   I   W   G   V   I   G   P   Q   R   Q   F
    TCA CAT GGG CAG ATT TAT TAT GGG TTA CTT TTC TTT TTC ATC ATT GGT GCT GTG ACC CCT GTC ATC AAT TGG TGG  2035
635 S   H   G   Q   I   Y   Y   G   L   L   F   F   F   I   I   G   A   V   T   P   V   I   N   W   L
    ATC TTG AAA AAA TGG CCA AAC TCT CCA GTC AAG TAT TTG CAT TGG CCA GTG TTC TTT TCT GGG ACA GGG TAC ATT  2110
660 I   L   K   K   W   P   N   S   P   V   K   Y   L   H   W   P   V   F   F   S   G   T   G   Y   I
    CCT CCA GCC ACT CCA TAT AAC TAT ACC TCC TAC TGT GCT GTG GGT TTG TTC TTT GGA TGG TGG ATT AAA AAG AAG  2185
685 P   P   A   T   P   Y   N   Y   T   S   Y   C   A   V   G   L   F   F   G   W   W   I   K   K   K
    TGG TTC CAC TGG TGG TCT AAA TAC AAC TAT TCC TTG TCT GCG GGC TTG GAT ATT GGT TTG GCA TGG TGC TGC TTG  2260
710 W   F   H   W   W   S   K   Y   N   Y   S   L   S   A   G   L   D   I   G   L   A   W   C   S   L
    ATC ATT TTC TTG TGC TTG AGT TTA ACA AAC ACC GAC TTC CCA TCG TGG TGG GGA AAC GAT GTG ATC AAC ACC ACT  2335
735 I   I   F   L   C   L   S   L   T   N   T   D   F   P   S   W   W   G   N   D   V   I   N   T   T
    CTC GAC ACT CAG GTT GTC ACC AAT ATC AGA CAC ATA TTG AAA GAG GGA GAG GCA TTT GGG CCA TCT TCC TGG TAA  2410
760 L   D   T   Q   V   V   T   N   I   R   H   I   L   K   E   G   E   A   F   G   P   S   S   W

    GCTGACGAAGAAACACACACACACACATTATTGCTTCCTATTGTCGTGTCTTTTATGTAGATGTAACGTGTTTTTATAAGAATGTAATTTAATTATTGT  2509
    ATATATATGCCAAATATTTTTACTGCCATTTTATATTCTTTCTGCCACTAAAAATGATAGGAGGTTTTGTATACTGGGTGTGCTTGTTTTACACGCGGG  2608
    CTCTTTTATTATTGATTTGAACAGTCTCTAAGGAAGTTACGAACTTATAGGTGAGTGCTAAAAATGAAAAGGGGAGTGAGGTTCCTTCTTATATCCTTT  2707
    TTGGCAAGTAAATGTGTCGTGCTTTGATATATTAGAAAGACAATCCATTAATAGATGAAATATATATTGATGATGAAAAAAGTATTGGTTGTTCAAAAT  2806
    GAAAGATCAATATAAAAATTCGGAGAGAAACGTGATGTTTATAGAGTAAAAAATTGAGCTGATAACTTCGCAACCAATTCTGAACAAGCATAGTTTGCA  2905
    AATATGAATACATCCTAGAAAAAGTGTAATCTATGAGGAAATATGCAGGATATTCATGATCTCTTAGCAAAATATTAAGGTTCAATCGGTTTTGTGATT  3004
    GGTTGCAAAATTTATCATTCGCGGTGTAAGTACACCAAGAAGTTAGACACCTACATGATCTTTTTTGTTTTTTCAATTCTTTTGATTTCCTATAAAAGT  3103
    TCTCCCGTTTTCCTTTCCTTTCTTTTCTGAAAATGAACAATATAGAATATCTTTTTTTGGTGAAATCACTGATTGCCTAACCTTCGTTCTTGAATTTAT  3202
    ATTCTATTAATATTGTTGTATCATCGCTGCTTCATTTCCATTCCTTCTGTTTCAAAATCAAATATAAAAAGTTCAAAGAATGATCAATAGTGACAATTT  3301
    TCAAGCTCGTGTAAACTGTGAAAGTAATACAAGACTCTGCAGAATACACATGCTGAAAATAATTAATGACAAAGGTATGTTGAACATGATC
```

FIG. 3c

```
Opt1       .......... .......... .......... .......... .......... ......MDki RaViSggEkp  14
Isp4       .......... .......... .......... .......... .......... .......... .mIgSInEsp   9
SCYJL212C  .......... .......... .......... .......... ......msti yresdsLEse psptpttipi  24
YSCP9677   msetvkdkvi idekvstkgt vdyaegaeys erlsnhssdf sqwytdeqil hfmkklgyen RtlydIpEdv  70
Consensus  ---------- ---------- ---------- ---------- ---------- ---------- R---SI-E--

Opt1       pVDtDNDhnt dFEadrKmpd lDIVVSKsqe .FDPvtshlv ndimEDEyaa V....hvED. ..........  68
Isp4       ieEhmNDsps TkEKadsVDI sDyIVShsDD sLskDikKdt KsflDvEhgE IstvdEFEE. ..........  68
SCYJL212C  qInMEeEkkd aFvKnideDV nnltattdEE drDPEsqKfd rhsiqEEgLv wKgdptYlp. ..........  83
YSCP9677   ayiLkkmpel TLEdsfKIlk dsIlyfKdDe niphDqyeew KrLvDlEdLD sKegiDeyDs fdirafasai 140
Consensus  -----ND--- TFEK--K-D- -DIIVSK-DE --DPD--K-- K---D-E-L- -K-----ED- ----------

Opt1       ..DSPYPEVR AAVPstDDPT lPqNTiRAWv iGLILttVGc GmNMLFSFhs PsfaitTFVt siLAWPIGnf 136
Isp4       ..DSPYPEVR AAVPPtDDPs mPcNTiRmWt iGLIYStVGA aVNMFFSLRn PtvtlsvLIs elLAYPalqI 136
SCYJL212C  ..nSPYPEVR sAVsieDDPT irlNhwRtWF lttVFvvVfA GVNqFFSLRy PSleiNfLVa QvvcYPIGrI 151
YSCP9677   kfhSPYqEVR AvVdPeDDPT iPveTfRAYF laiIWSvIGs GfNeFFShRv vSislNTpIi QmFlYicGka 210
Consensus  --DSPYPEVR AAVPP-DDPT -P-NT-RAWF -GLI-S-VGA GVNMFFSLR- PS---NTL-- Q-LAYPIG-I

Opt1       WAwIvPDWKI ....FgasLN PG.PFnVKEH tiITIManVS FgtGAAYATd IlLAQnMFYK SnFGWGYnLL 201
Isp4       WdLIfPDRef rIgrLKFnFk PG.PFnVKEH AlIvVMssVS F..GnAYsTd IiLAQrvhYK qrFGFGYeic 203
SCYJL212C  lAL.lPDWKc skvpF.FdLN PG.PFtkKEH AvVTI..AVa LtsstAYAmy IlnAQgsFYn mkLnvGYQFL 216
YSCP9677   WAktiPcWtI tIrgrKYgiN idkPWtqKEq mfsTlLyAIc ..qGAfYthy niLtQkLFYh SaFsFGYQFL 278
Consensus  WALI-PDWKI -I--FKF-LN PG-PF-VKEH A-ITIM-AVS F--GAAYAT- I-LAQ--FYK S-FGFGYQFL
```

FIG. 4a

FIG. 4

FIG. 4a
FIG. 4b
FIG. 4c

```
Opt1       LIwSTQcIGF aFgGvMRRFV VdspgAiWPl NLVTaTFLtn MHinE..Nht ANGWkiSRLa FFvIVFVASF 269
Isp4       LtlaTQLIGY GLAGlsRRLl VrPASmlWPv NLVqcTLiKt LHrKDlrNaV ANGWriSpFR FFLyVFIASF 273
SCYJL212C  LVwtsQMIGY GaAGltRRWV VnPASsiWPq tLIsVsLFds LHsrkvektV ANGWtmpRYR FFLIVLIgSF 286
YSCP9677   LslSvQFIGF GFAGiLRkFV VyPArAlWPt vMpTIainKa LlgKE..... khesgmSRYk FFFltFFimF 343
Consensus  L--STQ-IG- GFAG--RRFV V-PASA-WP- NLVT-TL-K- LH-KE--N-V ANGW--SRYR FFLIVFIASF

Opt1       VWyWFPGYIF QALSYFsWIT W..IKPNNVI INQVFGsSsG LGmiPnnIaL DWNQIa.gYI GSPLIpPasV 336
Isp4       IWnWSPsYIF QALSlFaWVT W..IRPTspt VNQIFGeStG isILP..mTF DWNQIS.AYI lSPLmaPada 338
SCYJL212C  IWyWvPGFlF tgLSYFNvIl WgsktrhNfI aNtIFGtqsG LGaLP..ITF DYtQVSqAms GSvFatPfYV 354
YSCP9677   IYnWFPtYIi niLntFNWmT W..IKPsNIn lanItGgvtG LGInPis.sF DWNvIS...f nSPLVyPfWs 407
Consensus  -STTY--SYG LSFA-I-AVI TH-ILYHGKD --A--KD--- ---------- ---------R ----PDIH-R

Opt1       iatIFgsIVL iFWIVVPAih YsNTWYsQYL PISStgsFDr fqqtYNVsKI iDhKtlSFne aeYkkYSPlF 406
Isp4       lmnILLgVIL FFWIVtPAln FtNTWYgdYL PISSsgiiDh fgNSYNVTrI Lt.KDatFdL DaYqnYSPIF 407
SCYJL212C  santYasVli FFvIVlPclY FtNTWYakYM PViSgstYDN tqNkYNVTKI Ln.eDySinL EKYkeYSPVF 423
YSCP9677   yltqYLgcIL aalIVI.AvY YsNymscQYL PIftnslYtN tghSFkVTeV LD.sDnkLdv kKYqsYSPpY 475
Consensus  ---IYL-VIL FFWIV-PA-Y --NTWY-QYL PISS---YDN --NSYNVTKI LD-KD-SF-L -KY--YSP-F

Opt1       LSTTFaiSYG LSFAsIlAtI THtIcFHGrD liAsLKa... .......... .........k ..EkPDVHnR 452
Isp4       MSTTYalaFG LSFAsItsVI fHvILYHGKE iydrLrD... .......... .........p ..paPDIHek 453
SCYJL212C  vpfsYllSYa LnFAaViAVf vHcILTHGKD ivAkFKD... .......... .........R knggtDIHmR 471
YSCP9677   ySagnlvSYG aficayplmI TwsfivHsKl lfnaFKDwal nlwamrklks wvtmfksdyR alDdyDdphs 545
Consensus  -STTY--SYG LSFA-I-AVI TH-ILYHGKD --A--KD--- ---------- ---------R ----PDIH-R

Opt1       LM.KaYKpVP EWWYLvVflV FFGMsIATVr aWPTEmPVWG LVfAlIiaiI FLlPValiyA kTNIavGLNV 521
Isp4       LM.KaYdEVP fYWYLsVFla FFGMmmgTIY gWkTETPwwv iIVgvIfSaV WFIPIGIvQA iTNIqlGLNV 522
SCYJL212C  iysKnYKDcP DWWYLllqiV miGLGfvaVC cFdTkfPaWa FVIAiliSLV nFIPqGIleA mTNqhvGLNI 541
YSCP9677   namKnYKEVP DWWYFaILig sLvvgIAvVe hYPTnTPVWG LfVclgfnFV FLIPttIlQA tTgysfGLNl 615
Consensus  LM-K-YKSVP DWWYL-VF-V FFGMGIATV- -WPTETPVWG LVVA-I-S-V F-IP-GI-QA -TNI--GLNV
```

FIG. 4b

```
Opt1        vTEFIVGYvL gGRPLcmmlF TKFGYItnnQ avtFVqDMKL gHYMKIdPRt LFWaQfaATI wgsLVqIaVL 591
Isp4        fTEFIVGYMy PGRPLAMMiF KTvGYItMtQ gLaFaaDLKF gHYMKlPPRi MFYtQmIATI wScFVqIGVL 592
SCYJL212C   iTELlcGYML PlRPMAnLlF KlYGFIvMrQ gLNLsrDLKL amYMKVsPRl iFavQiyATI iSgMVnVGVq 611
YSCP9677    liEMvmGYaL PGnPiAiMiL KaFGYniDGq adNYVsnLKi aHYcKIPPma LFRgQcVivf iqiFVnlGVL 685
Consensus   -TEFIVGYML PGRPLAMM-F KTFGYI-M-Q -LNFV-DLKL -HYMKIPPR- LF--Q--ATI -S-FV-IGVL

Opt1        EWAyGaIDNL CaAdQkNhYT CPNgkvFFNA SIIWGVIGPq RqFShGqIYy GLLFFFiIGA vtPVinWlIl 661
Isp4        DWALGNIDNv CqADQPdnYT CPNatvFFNs SVIWGVIGPK RMFSgkntYt GLqYFWLaGv LgtILfWAlW 662
SCYJL212C   EWmMhNIDgL CTtDQPNgFT CaNgrtvFNA SIIWsl..PK yLFSsGrIYn pLMWFFLIGl LfPlavYAVq 679
YSCP9677    nWqisNIkdF CTphQnakFT CPdavtYYNA SVVWGaIGPK RiFny..IYp iFkWcWLIGA cigIF.FgVW 752
Consensus   EWA-GNIDNL CTADQPN--T CPN---FFNA S-IWGVIGPK R-FS-G-IY- GL-WF-LIGA L-PI--WAVW

Opt1        KKWPn.spvK yLHwPvFFsG TGYIPPaTPY NYtsYcA... VgLFFgWWiK KkWfhWWsKY NYsLSAGLDi 727
Isp4        KKWPq.KWWg qLngPliFgG TGYIPPaTPv NYLaWsg... IgLFFNYYlK KiFadWWqKY NFtLSA.LDT 727
SCYJL212C   wKFPkfKFak hiHtPvFFtG pGnIPPsTPY NYslFfA... msFcLN.lir KrWraWFnKY NFVMgAGvEa 745
YSCP9677    KrWg..KFYp ryfdPmLFvG .GmlnmspPy NlMyYtsgmi VsYisqYYmK rhhLnlWeKY NYVLSAGFsT 819
Consensus   KKWP--KF-K -LH-P-FF-G TGYIPP-TPY NY--Y-A--- V-LFFNYY-K K-W--WW-KY N-VLSAGLDT

Opt1        GLAwcslIIF LClsltnTdF PSWWGNDVin tTLDtqvvtn IRhiLKEGEa FGpssW...  783
Isp4        GtqLSVlIlF FClQlPmvnF PdWWGNDgaF nTLDa.tgaa VRklvnEsar .........  776
SCYJL212C   GvAiSVVIIF LCvQyPggkL .SWWGNnVwk rTyDndykkF y..TLKkGEt FGYdkWw..  799
YSCP9677    GLvLSaIIIF FavQykdTaF .nWWGNtVpY agaDgvgypL kniTdtangy FGYapghyp  877
Consensus    GLALSVIIIF -C-Q-P-T-F PSWWGNDV-- -TLD------ -R-TLKEGE- FGY--W---
```

FIG. 4c

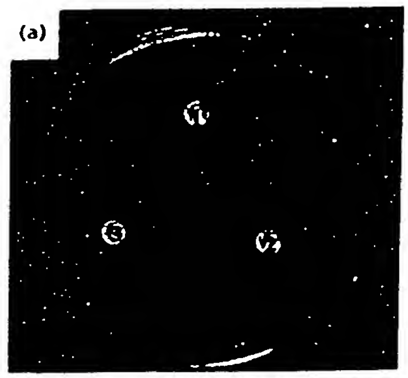

FIG. 5a

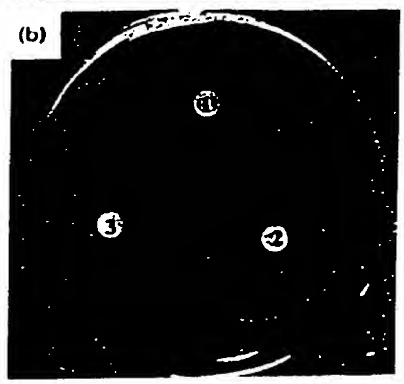

FIG. 5b

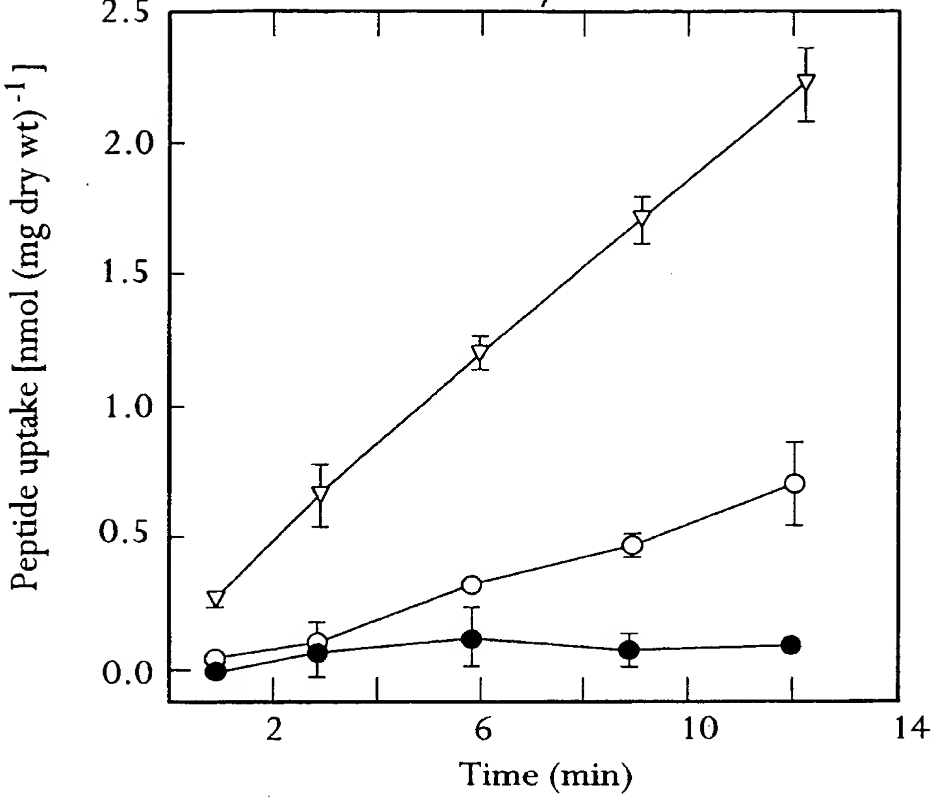
2.5
2.0
1.5
1.0
0.5
0.0
Peptide uptake [nmol (mg dry wt)$^{-1}$]
2
6
10
14
Time (min)

FIG. 6a

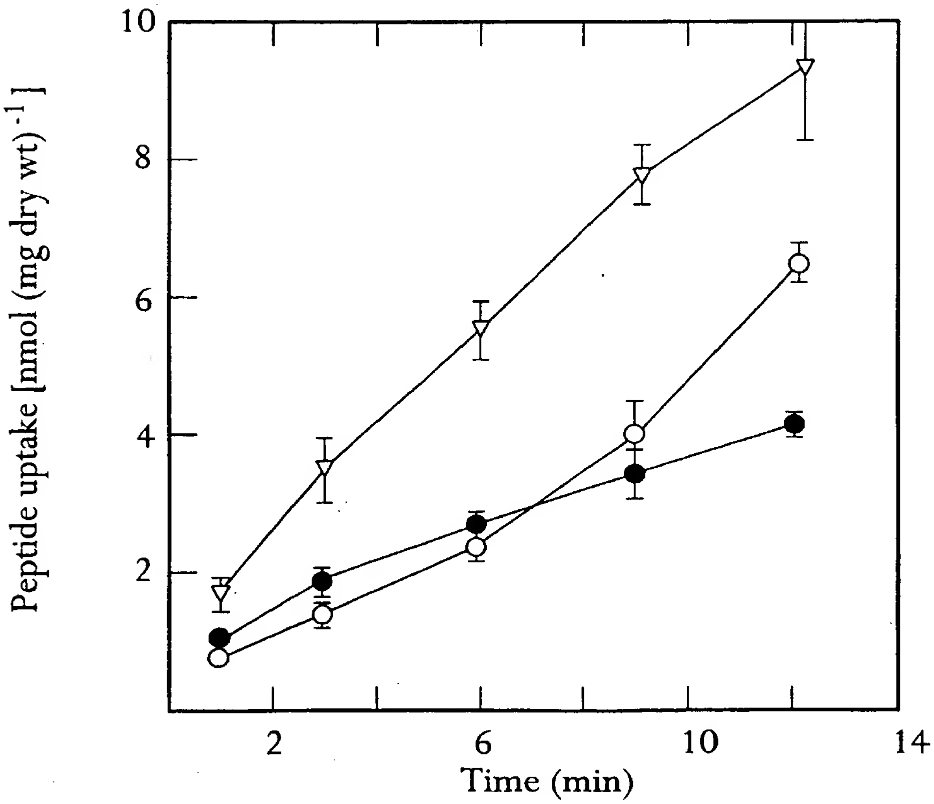
10
8
6
4
2
Peptide uptake [nmol (mg dry wt)$^{-1}$]
2
6
10
14
Time (min)

FIG. 6b

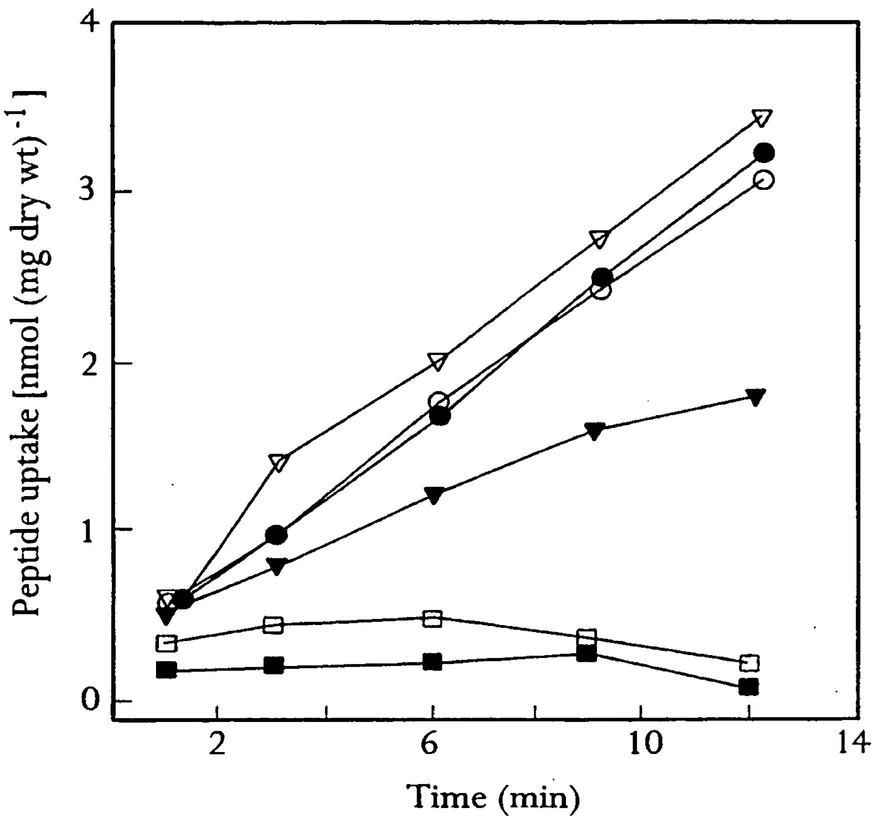

FIG. 7

INTERNATIONAL SEARCH REPORT	International application No. PCT/US98/02332

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 14/40; C12N 15/31
US CL : 435/69.1, 252.3; 530/350; 536/23.74
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 252.3; 530/350; 536/23.74

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN/MEDLINE
search terms: transporter#, candida, oligopeptide#

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- A	FEI et al. Expression cloning of a mammalian proton-coupled oligopeptide transporter. Nature. 07 April 1994, Vol. 368, pages 563-566, especially Figure 1 on page 563.	1, 3-5, 15-20 --------- 2, 6-14, 21-24
X --- A	FLING et al. Analysis of a Candida albicans gene that encodes a novel mechanism for resistance to benomyl and methotrexate. Molecular & General Genetics. 1991, Vol. 227, pages 318-329, especially Figure 3 on pages 322 and 323.	1-5, 15-21 --------------- 6-14, 22-24

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search	Date of mailing of the international search report
09 APRIL 1998	26 MAY 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer JOHN D. ULM
Facsimile No. (703) 305-3230	Telephone No. (703) 308-4008